

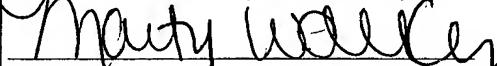
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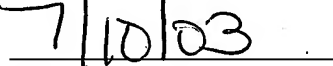
In re Application of: MURDO BLACK ET AL.
Filed: HERewith
For: ENZYME ELECTRODES AND METHOD OF
MANUFACTURE

Serial No.: UNKNOWN
Priority Date Claimed: JULY 11, 2002
Group Art Unit: UNKNOWN
Examiner: UNKNOWN
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REQUEST FOR PRIORITY UNDER 35 USC 119

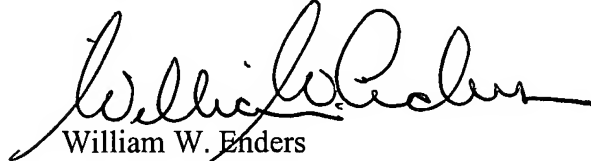
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Sir:

In the matter of the above-identified application for patent, notice is hereby given that the Applicant claims as the priority date July 11, 2002, the filing date of the corresponding application filed in United Kingdom, application number 0216039.8.

The Certified copy of the corresponding Convention Application is enclosed herewith.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "William W. Enders", written in a cursive style.

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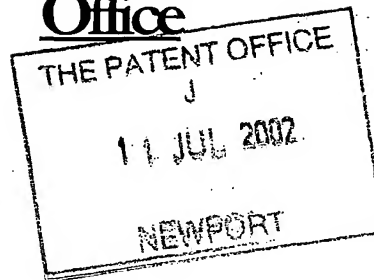
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1.	Your reference	P3363.A4/PAG		
2.	Patent application number (The Patent Office will fill in this part)	0216039.8	11JUL02 E732509-4 001070 P01/7700 0.00-0216039.8	
3.	Full name, address and postcode of the or of each applicant (underline all surnames)	Hypoguard Limited Dock Lane Melton Woodbridge IP12 1PE		
	Patents ADP number (if you know it)	7309644001		
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom		
4.	Title of the invention	Enzyme Electrodes and Method of Manufacture		
5.	Name of your agent (if you have one)	DUMMETT COPP		
	"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	25 THE SQUARE MARTLESHAM HEATH IPSWICH IP5 3SL		
	Patents ADP number (if you know it)	6379001		
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day / month / year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day / month / year)	
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	Yes		

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Description 18
Claim(s) 8
Abstract 1
Drawing(s) 10 + 0

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Priority documents

Translation of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) ONE

Request for substantive examination (Patents Form 10/77) ONE

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

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Date

9 July 2002

12. Name and daytime telephone number of person to contact in the United Kingdom Peter Gemmell
01473 660600

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ENZYME ELECTRODES AND METHOD OF MANUFACTURE

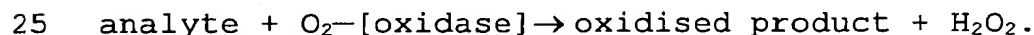
BACKGROUND OF THE INVENTION

5 1. Field of the Invention

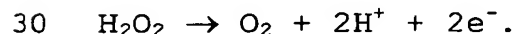
The present invention relates to enzyme electrodes for measuring analyte concentration in fluids, for example glucose in whole blood. Enzyme electrodes comprise an enzyme layered on or mixed with an electrically conductive substrate. The electrodes respond amperometrically to the catalytic activity of the enzyme in the presence of a suitable analyte (substrate). The invention also extends to a biosensor, notably a single-use biosensor, which includes the enzyme electrode.

2. Description of the Prior Art

Amperometric biosensors are well known in the art. Typically the enzyme is an oxidoreductase, for example glucose oxidase, cholesterol oxidase, or lactate oxidase, which produces hydrogen peroxide according to the reaction:



The peroxide is oxidised at a fixed-potential electrode as follows:



Electrochemical oxidation of hydrogen peroxide at the platinum centres on the electrode results in transfer of electrons from the peroxide to the electrode producing a current which is proportional to the analyte concentration. Where glucose is the analyte, the oxidised product is gluconolactone. Japanese Unexamined Patent Publication No. 56-163447 describes a system which employs glucose oxidase immobilised on a platinum electrode. The electrode comprises a layer of immobilised enzyme on an electrically conductive carbon base. The base is formed from moulded graphite containing up to 10 parts by weight of a fluorocarbon resin binder, onto which is deposited a thin (less than 1 μm) platinum film. The invention is said to avoid the problems associated with the immobilisation of the enzyme directly onto the platinum surface and to produce an enzyme electrode having rapid response times (5 seconds), high sensitivity and durability. However, according to US Patent No. 4,970,145, recent experimental work with such electrodes has failed to elicit such benefits.

US Patent No. 4,970,145 describes an enzyme electrode comprising a substantially heterogeneous porous substrate consisting essentially of resin-bonded carbon or graphite particles with a platinum-group metal dispersed substantially uniformly throughout the substrate, and a catalytically active quantity of an enzyme adsorbed or immobilised onto the surfaces of the porous substrate. The electrodes are manufactured either by cross-linking

- the enzyme to the substrate, or by suspending the porous substrate in a buffered solution of the enzyme for 90 minutes at room temperature. Alternatively, adsorption of the enzyme to the electrode is effected by
- 5 electroadsorption, wherein the electrode base material is suspended at a positive potential in an enzyme solution for 60 minutes. The electrode is said to have fast response times (1-2 seconds without a protective membrane, and 10 to 30 seconds with a membrane) and good stability.
- 10 The working range is said to be extended, and the electrode requires a substantially lower operating potential than normal (325 mV against the more usual 650 mV) and exhibits low background at the operating potential.
- 15 US Patent No. 5,160,418 discloses a simplified enzyme electrode comprising a thin film of a substantially homogeneous blend of enzyme and finely-divided platinum group metal or oxide. Optionally, platinised or
- 20 palladised finely-divided carbon or graphite may be used and, also optionally, a binder. The film can be made by screen-printing a liquid suspension containing the components.
- 25 Desirable attributes for a single use biosensor include:
- low intercept, related to background - to achieve low coefficients of variation (CV's) after calibration;
 - as high a sensitivity as the electronics will
- 30 allow;

stability;

- good precision;
- reproducible manufacture;
- rapid response;
- 5 • low cost.

We have found that prior art systems such as described above have high intercepts relative to sensitivity, resulting in poor calibrated precision. We have also
10 found that there is a gradual attenuation of sensitivity with time which is not necessarily related to enzyme instability.

The present invention seeks to provide an enzyme electrode
15 and biosensor which are improved in respect of at least some of the above criteria.

SUMMARY OF THE INVENTION

20 According to an aspect of the present invention there is provided an enzyme electrode for indicating amperometrically the catalytic activity of an enzyme in the presence of a fluid containing a substance acted upon by said enzyme and of an electric potential on the
25 electrode, said electrode comprising a base substrate on which is provided:

- (a) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
- 30 (b) a top layer on the base layer, said top layer

comprising a buffer; and

(c) a catalytically active quantity of said enzyme in at least one of said base layer and said top layer.

- 5 We have found that by providing the buffer in the top layer, we can get faster response times than conventional non-mediated biosensors, together with increased stability and sensitivity. The increase in sensitivity and response time we believe is achieved by providing a high buffering capacity on the strip. The oxidation of hydrogen peroxide produces hydrogen ions which are neutralised by the buffer. This can have two effects: it sustains enzyme activity by maintaining the local pH around the enzyme, and it also shifts the equilibrium of the hydrogen peroxide oxidation making it more efficient. Improving the efficiency of hydrogen peroxide oxidation also results in greater oxygen production which can be utilised by the oxidoreductase enzyme.
- 10
- 15
- 20 The pH range for the buffer will depend on the specific chemistry of the system. A preferred range is pH 7-10, notably 7 to 8.5. A preferred buffer is phosphate, at about pH 8.
- 25 The platinum group metal or oxide may be present in sufficient quantity for the base layer to be electrically conductive, as taught in US 5,160,418. Alternatively, the base layer may also contain particles of finely divided carbon or graphite. For convenience, the term "catalyst" will be used herein to refer to the finely divided
- 30

platinum group metal or oxide. In a preferred embodiment, the catalyst is in intimate surface contact with the carbon or graphite particles, for example as platinised carbon or palladised carbon.

5

The enzyme electrode may be manufactured by printing an ink containing the catalyst on the base substrate, allowing the printed ink to dry to form a base layer, and subsequently forming the top layer by coating a fluid
10 containing the buffer onto the base layer. Suitable methods for forming the top layer include printing, spraying, ink jet printing, dip-coating or spin coating. A preferred coating technique is drop coating.

15 Typically, the enzyme electrode will be incorporated in the working electrode of a biosensor, and a reference electrode will also be provided for completing a circuit and providing a stable reference potential, as is well known in the art.

20

Accordingly, a further aspect of the invention provides a biosensor for indicating amperometrically the catalytic activity of an enzyme in the presence of a fluid containing a substance acted upon by said enzyme, the
25 biosensor comprising:

- (a) a base substrate;
- (b) a working electrode and a reference electrode on the base substrate;
- (c) conductive tracks connected to the said electrodes
30 for making electrical connections with a test meter

apparatus;

wherein the working electrode includes:

- (d) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
- (e) a top layer on the base layer, said top layer comprising a buffer; and
- (f) a catalytically active quantity of said enzyme in at least one of said base layer and said top layer.

In a preferred embodiment, the enzyme is provided in the top layer with the buffer. A system stabiliser may advantageously be included in the top layer. Suitable stabilisers include polyols other than those which are acted upon by the enzyme; for example trehalose, mannitol, lactitol, sorbitol or sucrose where the enzyme is glucose oxidase. The system stabiliser may stabilise the enzyme by encapsulation, hindering tertiary structural changes on storage, or by replacing the water activity around the enzyme molecule. The glucose oxidase enzyme has been shown to be a very stable enzyme and the addition of stabilisers are not primarily to protect this enzyme. The stabilisers help to reduce long term catalyst passivation effects, for example by coating a platinised carbon resin base layer as well as blocking the carbon surface to air oxidation.

If carbon particles are present in the base layer, a blocking agent may optionally be included in that layer to block active sites on the carbon particles. This aids shelf stability and uniformity of the carbon's activity. Suitable blocking agents include the system stabilisers

and also proteins, for example bovine serum albumin (BSA). If graphite particles are used instead of high surface carbon, the particles have higher conductivity, and a blocking agent is less desirable because the number of active moieties on the graphite is much less than that found on carbon. The smaller surface area and less active surface groups both tend to reduce the intercept. At 0 mM of analyte the intercept consists mainly of a capacitative component which is surface area related.

10

The base substrate may be formed from any suitably heat-stable material. Heat stability is important to ensure good registration of prints in the manufacturing process. A preferred substrate is Valox FR-1 thermoplastic

15

polyester film (poly(butylene terephthalate) copoly (bisphenol-A/tertabromobisphenol-A-carbonate). Other suitable substrates will be well known to those skilled in the art, for example PVC, poly (ether sulphone) (PES), poly (ether ether ketone) (PEEK), and polycarbonate.

20

Other aspects and benefits of the invention will appear in the following specification, drawings and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be further described, by way of example, with reference to the following drawings in which:

5

Figure 1 shows stages in the formation of a biosensor in accordance with an aspect of the invention;

10

Figure 2 is a graph illustrating a comparison between glucose calibrations on a biosensor of the present invention and a prior art biosensor;

15

Figure 3 is a graph illustrating the effect of phosphate buffer in the top layer on response;

Figure 4 is a graph illustrating the effect of pH on glucose response;

20

Figures 5 and 6 are graphs illustrating the effect of pH on hydrogen peroxide response;

Figures 7 and 8 are graphs illustrating the effect of pH on glucose response; and

25

Figures 9 and 10 are graphs illustrating the effect of buffer type on hydrogen peroxide response.

DETAILED DESCRIPTION

Preparation of BSA-Pt/Carbon

5 In a 250 ml glass bottle, 6.4 g of BSA, Miles Inc. was
dissolved in 80 ml of phosphate buffered saline (PBS) and
20 g of 10%Pt/XC72R carbon, MCA Ltd, was gradually added
with constant stirring. The bottle was then placed on a
roller mixer and allowed to incubate overnight at room
10 temperature.

A Buchner funnel was prepared with two pieces of filter
paper, Whatman™ No 1. The mixture was poured into the
funnel and the carbon washed three times with
15 approximately 100 ml of PBS. The vacuum was allowed to
pull through the cake of carbon for about 5 minutes to
extract as much liquid as possible. The cake of carbon was
carefully scraped out into a plastic container and broken
up with a spatula. The carbon was then placed in an oven
20 at 30°C overnight to dry. The purpose of this procedure is
to block active sites on the carbon hence to aid the shelf
stability and reproducibility of the carbon's properties.

Preparation of platinum group metal/carbon inks

25 BSA-Pt/Carbon was prepared in Metech 8101 polyester resin
as the polymer binder, Terpeneol BP, from RC Treatt, as a
flow agent and Butyl Cellosolve Acetate (BCA) as a solvent
for the ink.

30

The formulation of the ink consisted of:

	Metech 8101 resin	54.05%
	BSA-Pt/Carbon	27.09%
	BCA	12.57%
5	Terpineol BP	6.29%

The resin, solvent and flow agent were initially blended together prior to adding the carbon fraction. Initially the formulation was hand mixed followed by several passes
10 through a triple roll mill. This produces a smooth homogeneous thixotropic carbon ink suitable for screen-printing.

An alternative formulation is one similar to that
15 described in US 4,970,145 in which glucose oxidase (GOD) is adsorbed onto the Pt/Carbon prior to BSA adsorption and incorporation into an ink.

20 Preparation of Drop Coating Solution

The coating solution consists of a high concentration of buffer, preferably phosphate at pH 8. It has been found that buffering capacity is more important than ionic strength. In this example the solution contains glucose
25 oxidase and a system stabiliser, in this example trehalose.

	Buffer	$\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$	385 mM, pH 8	Sigma
	Enzyme	Glucose oxidase	4080 U/ml	Biozyme
30	Stabiliser	Trehalose	1%	Sigma

Preferred Ranges

5

Buffer	100 - 500 mM, pH 7 - 10
Enzyme	500 - 12000 U/ml
Stabiliser	0.5 - 10%

- 10 If the enzyme is located on the platinised carbon particles, the drop coating solution may contain only buffer, optionally with the stabiliser.

Method of Manufacture

15

Glucose test strips (biosensors) were manufactured using a combination of screen printing and drop coating technologies. Other printing and/or coating technologies, well known per se to those skilled in the printing and
20 coating arts may also be used.

With reference to Figure 1, a base substrate 2 is formed from a polyester (Valox™). Conductive tracks 4 were printed onto the substrate 2 as a Conductive Carbon Paste, product code C80130D1, Gwent Electronic Materials, UK. The
25 purpose of this ink is to provide a conductive track between the meter interface and the reference and working electrodes. After printing, this ink was dried for 1 minute in a forced air drier at 130°C. The second ink
30 printed on top of the conductive carbon 4 is a

Silver/Silver Chloride Polymer Paste, product code C61003D7, Gwent Electronic Materials, UK. This ink 6 is not printed over the contact area or the working area. This ink 6 forms the reference electrode 16 of the system.

5 It is dried at 130°C in a forced air drier for 1 minute. The next layer is the platinum group metal carbon ink (formulated in this example as set forth above) which is printed as a layer 8 onto the conductive carbon 4 in the target area. This ink is dried for 1 minute at 90°C in a

10 forced air drier. A first dielectric layer 10 is then printed. The first dielectric layer 10 is MV27, from Apollo, UK. The purpose of this layer is to define a target area for blood application and to insulate the system. It is dried at 90°C for 1 minute in a forced air

15 drier. A spreading layer 12, which consists of a surfactant-coated polyester mesh, Saaticare PES 105/52, Saati, Italy, is then placed over the target area. This is then sealed onto the electrode using a further layer 14 of MV27 dielectric and dried. If desired, the base layer 8

20 can alternatively be printed after the first dielectric layer 10. However, it is preferred to print the base layer 8 first, since the subsequent application of the first dielectric layer 10 removes some of the tolerance requirements of the print.

25

The drop coat layer, formulated in this example as described earlier, is then applied to the electrode using BioDot drop coating apparatus. The volume of drop coating solution used is 1 µl; this is dried in a forced air drier

30 for 1 minute at 50°C. The final biosensor 20 has a

reference electrode 16 and a working electrode 18 within the target area. The working electrode comprises the base layer 8 on a conductive carbon layer 4 on the base substrate 2, and a top layer including the buffer. The mesh 12 helps to spread out a sample of blood when this is applied to the target area.

Preparation of Comparative Biosensor (Prior Art)

10 An ink was formulated as described above (Preparation of platinum group metal/carbon inks) but using glucose oxidase (GOD) in place of BSA. The ink was used in the manufacture of a biosensor as described above (Method of Manufacture) but without the drop coating step.

15

Standard Test Procedures

The test procedure involves connecting the test strips to a potentiostat. A potential of 350 mV is applied across the working and reference electrodes after application of a sample, in this example a sample of whole blood. The potential is maintained for 15 seconds, after which the current is measured; this current is used to prepare response graphs.

25

Explanation of Figures

- Comparison between glucose calibrations on old and new methodologies (Fig. 2). The old methodology refers to the prior art non-mediated Comparative Biosensor, which does not include high buffer

30

concentrations. It can be seen that increasing the pH from pH 7.4 within the ink at low buffer concentrations to pH 8 at a high local buffer concentration with a top layer which also includes the enzyme results in a dramatic increase in sensitivity.

- **Effect of Phosphate buffer concentration, in top layer, on response (Fig. 3).** It can be seen in this plot that the sensitivity of the response to glucose and also to hydrogen peroxide is dramatically increased by the concentration of phosphate buffer. The buffer pH was pH 7.4. This plot also demonstrates the efficiency gap between hydrogen peroxide measurement and glucose measurement. Hydrogen peroxide is being directly oxidised at the platinum surface whereas glucose has to react with glucose oxidase and produce hydrogen peroxide. Where glucose oxidase is in the top layer it rapidly comes into solution on application of sample diffusing into the bulk. Hydrogen peroxide produced by glucose oxidation has a variable diffusion distance to the electrode surface whereas hydrogen peroxide applied in the sample does not. The ideal situation would be to have glucose oxidase immobilised at the electrode surface yet have high ionic strength and stabilisers in the top layer.

Effect of pH on glucose response (Fig. 4). This

plot shows that the glucose response is increased with pH. The buffer concentration at each pH is maintained at 350 mM. This cannot be due to increased activity from the glucose oxidase as it has its pH maximum at pH 5.6; at pH 10 the glucose oxidase should be grossly inhibited. A possible mechanism for the increased glucose related response is that the glucose is being directly oxidised at the electrode surface. It is known that glucose will oxidise at a platinum surface but under normal conditions this response should be very small in comparison with the enzyme facilitated glucose oxidation. It may be that high buffer concentration coupled with high pH results in a huge enhancement in the direct oxidation of glucose, although this is unlikely. Alternatively if the hydrogen peroxide response were to increase with pH this might compensate for some of the drop-off in glucose oxidase response.

- **Effect of pH on hydrogen peroxide response (Fig. 5, Fig. 6).** This plot shows very little change in hydrogen peroxide response with pH, except at low hydrogen peroxide concentrations. The sensitivity to hydrogen peroxide is a factor of five higher than that to glucose hence it is more applicable to look at low hydrogen peroxide concentrations. Increased pH will result in ionisation of active groups on the carbon surface, this increases the non-Faradaic component of the electrochemical

response resulting in an increased intercept.

- **Effect of buffer type on glucose response (Fig. 7, Fig. 8).** Different buffers were evaluated. All buffers were drop coated on the electrode surface and dried. All buffers were at pH 7.4 and 350 mM. The buffers could be separated into 3 distinct groups.

5
10
group A - bis-tris - this buffer resulted in a high intercept and relatively poor sensitivity to glucose.

group B - phosphate, MOPS, MES, HEPES, ACA, ACES, TES and Tricine - these buffers all gave roughly similar responses, low intercepts and reasonable sensitivity to glucose.

15
group C - Borate, Tris - these buffers gave low intercepts but poor glucose sensitivity.

- **Effect of buffer type on hydrogen peroxide response (Fig. 9, Fig. 10).** Similar electrodes to that used for the effect of buffer type on glucose response were used in this experiment. The buffer types could be separated into 3 groups.

20
25
group A - bis-tris - this buffer resulted in a high intercept but reasonable sensitivity above 4 mM.

group B - phosphate, MOPS, HEPES, ACES, TES, ACA - all gave similar responses low intercept and reasonable sensitivity to hydrogen peroxide.

30
group C - Borate, Tris and Tricine - low intercepts and reduced sensitivity to hydrogen peroxide

There are similar trends when comparing the glucose and hydrogen peroxide sensitivities with buffer type. This would imply that the major effect of the buffer is on hydrogen peroxide oxidation. The bis-tris buffer is electrochemically active which results in the high background current at zero hydrogen peroxide. The Borate Tris and Tricine buffers have pKa values greater than 8, hence they would have poor buffering capacity at pH 7.4. All the other buffers have pKa values close to 7.4.

CLAIMS

1. An enzyme electrode for indicating amperometrically the catalytic activity of an enzyme in the presence of a fluid containing a substance acted upon by said enzyme and of an electric potential on the electrode, said electrode comprising a base substrate on which is provided:
 - (a) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
 - (b) a top layer on the base layer, said top layer comprising a buffer; and
 - (c) a catalytically active quantity of said enzyme in at least one of said base layer and said top layer.
2. An enzyme electrode according to claim 1, wherein the buffer is selected from a group comprising: phosphate, MOPS, MES, HEPES, ACA, and ACES, or buffers with a pK_a 7.4 ± 1 .
3. An enzyme electrode according to claim 1 or claim 2, wherein the buffer has a pH in the range 7 to 10.
4. An enzyme electrode according to claim 3, wherein the buffer has a pH in the range 7 to 8.5.
5. An enzyme electrode according to any preceding claim, further including a system stabiliser in the top layer, comprising a polyol which is not acted upon by the enzyme.

6. An enzyme electrode according to claim 5, wherein the system stabiliser is selected from trehalose, mannitol, and sucrose.
- 5 7. An enzyme electrode according to claim 6, wherein the system stabiliser is trehalose.
8. An enzyme electrode according to any preceding claim, wherein the base layer also contains particles of finely-
10 divided carbon or graphite.
9. An enzyme electrode according to claim 8, wherein said finely divided particles of platinum group metal or oxide are preadsorbed onto the surface of the finely-
15 divided carbon or graphite.
10. An enzyme electrode according to claim 8 or claim 9, wherein the particles of finely divided carbon or graphite comprise carbon, and wherein the base layer further
20 includes a blocking agent for blocking active sites of the carbon particles.
11. An enzyme electrode according to claim 10, wherein the said blocking agent comprises a protein or a polyol.
25
12. An enzyme electrode according to claim 11, wherein the blocking agent is bovine serum albumin (BSA) or trehalose.
- 30 13. An enzyme electrode according to any preceding claim,

wherein the said enzyme is located substantially in the said top layer.

14. An enzyme electrode according to any preceding claim,
5 further including a spreading layer for aiding spreading of the said fluid.

15. An enzyme electrode according to claim 14, wherein
the spreading layer is a surfactant-coated mesh formed
10 from a polymeric material.

16. An enzyme electrode according to claim 15, wherein the polymeric material is polyester or nylon.

15 17. A method for manufacturing an enzyme electrode for indicating amperometrically the catalytic activity of an enzyme in the presence of a fluid containing a substance acted upon by said enzyme and of an electric potential on the electrode, the method comprising the steps of:

20 (a) printing on a base substrate an ink containing finely divided platinum group metal or oxide and a resin binder;
(b) causing or permitting the said printed ink to dry to form an electrically conductive base layer comprising the said platinum group metal or oxide bonded together by the
25 resin;

(c) coating the base layer with a coating fluid containing a buffer; and

(d) causing or permitting the said coating fluid to dry to form a top layer on the base layer; wherein

30 (e) a catalytically active quantity of the said enzyme is

provided in at least one of the printed ink and the coating fluid.

18. A method according to claim 17, wherein the coating
5 fluid is applied by drop coating.

19. A method according to claim 16 or claim 17, wherein the said enzyme is provided in the coating fluid.

10 20. A biosensor for indicating amperometrically the catalytic activity of an enzyme in the presence of a fluid containing a substance acted upon by said enzyme, the biosensor comprising:

- (a) a base substrate;
- 15 (b) a working electrode and a reference electrode on the base substrate;
- (c) conductive tracks connected to the said electrodes for making electrical connections with a test meter apparatus;

20 wherein the working electrode includes:

- (d) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
- (e) a top layer on the base layer, said top layer
25 comprising a buffer; and
- (f) a catalytically active quantity of said enzyme in at least one of said base layer and said top layer.

21. A biosensor according to claim 20, wherein the buffer
30 is selected from a group comprising: phosphate, MOPS, MES,

(c) causing or permitting the said printed ink to dry to form an electrically conductive base layer comprising the said platinum group metal or oxide bonded together by the resin;

5 (d) coating the base layer with a coating fluid containing a buffer; and

(e) causing or permitting the said coating fluid to dry to form a top layer on the base layer; wherein

(f) a catalytically active quantity of the said enzyme is
10 provided in at least one of the printed ink and the coating fluid.

28. A method according to claim 27, wherein the coating fluid is applied by drop coating.

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29. A method according to claim 27 or claim 28, further including the step of applying a spreading layer on the base layer prior to application of the coating fluid.

20 30. A method according to claim 29, wherein the step of applying a spreading layer comprises applying a surfactant-coated polyester mesh.

31. A method according to claim 29, further comprising
25 the step of applying a first dielectric layer prior to applying the spreading layer, the first dielectric layer being applied around the reference electrode and the working electrode to define a target area to which the said fluid containing a substance acted upon by the enzyme
30 will be applied.

32. A method according to claim 31, further comprising the step of applying a second dielectric layer around the target area so as to secure the spreading layer in place.

5

33. A method according to any of claims 27 to 32, wherein the said enzyme is provided in the coating fluid.

34. An enzyme electrode for indicating amperometrically the catalytic activity of glucose oxidase in the presence of whole blood and of an electric potential on the electrode, said electrode comprising a base substrate on which is provided:

- 10 (a) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
- 15 (b) a top layer on the base layer, said top layer comprising a buffer; and
- 20 (c) a catalytically active quantity of glucose oxidase in at least one of said base layer and said top layer.

35. A biosensor for indicating amperometrically the catalytic activity of glucose oxidase in the presence of whole blood, the biosensor comprising:

- 25 (a) a base substrate;
- (b) a working electrode and a reference electrode on the base substrate;
- (c) conductive tracks connected to the said electrodes for making electrical connections with a test meter
- 30 apparatus;

- (d) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;

wherein the working electrode includes:

- 5 (e) a top layer on the base layer, said top layer comprising a buffer; and
- (f) a catalytically active quantity of said enzyme in at least one of said base layer and said top layer.

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ABSTRACT

ENZYME ELECTRODES AND METHOD OF MANUFACTURE

5 An enzyme electrode comprises a base substrate (2) on
which is provided an electrically conductive base layer
(8) comprising finely divided platinum group metal or
oxide bonded together by a resin; a top layer on the base
layer (8), the top layer comprising a buffer. A
10 catalytically active quantity of the enzyme is provided in
at least one of the base layer and the top layer. The
invention also provides a biosensor (20) which includes an
enzyme electrode, and methods of manufacturing the enzyme
electrode and biosensor.

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Figure 1.

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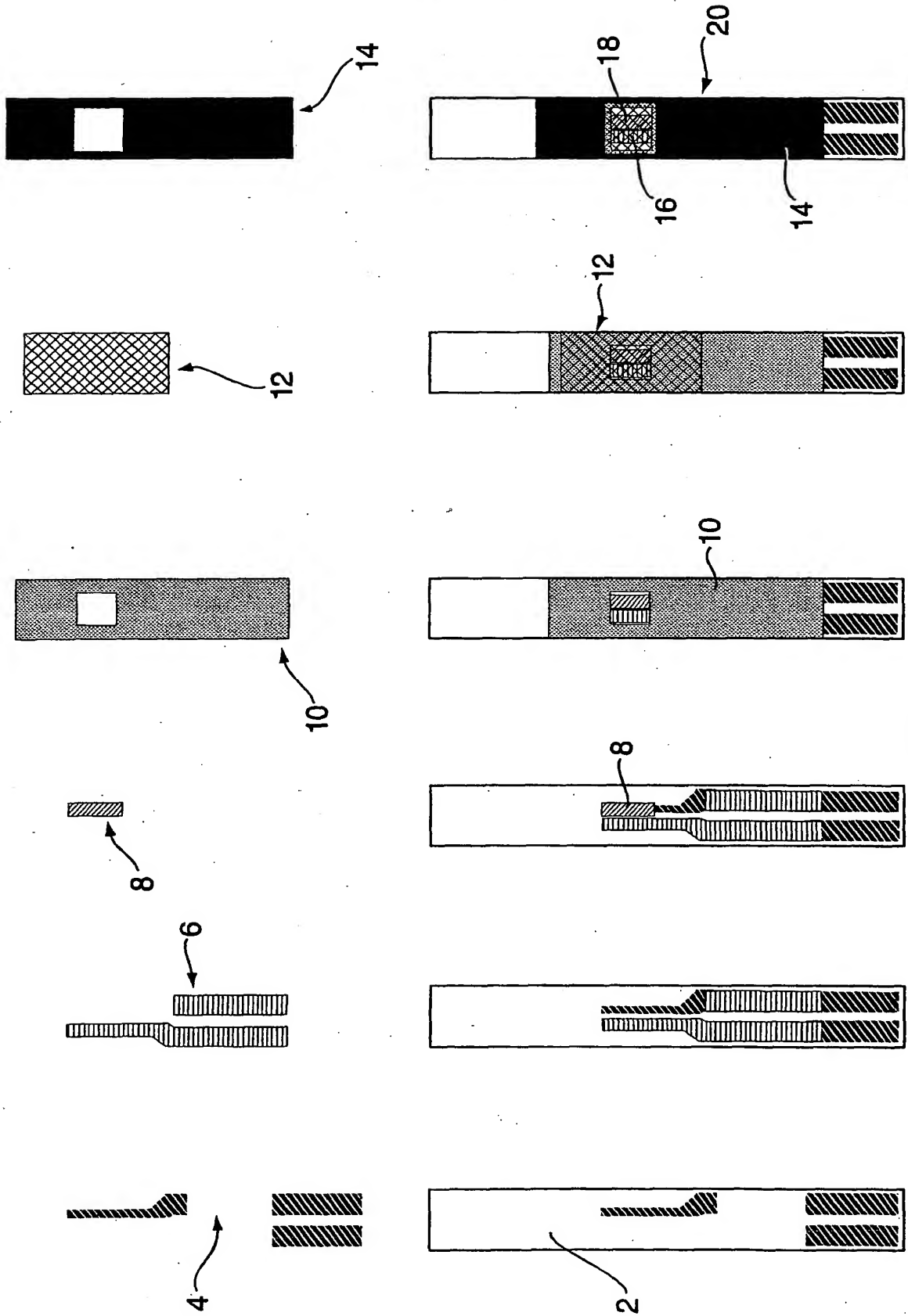


FIG. 1

Comparison between glucose calibrations on old and new methodologies

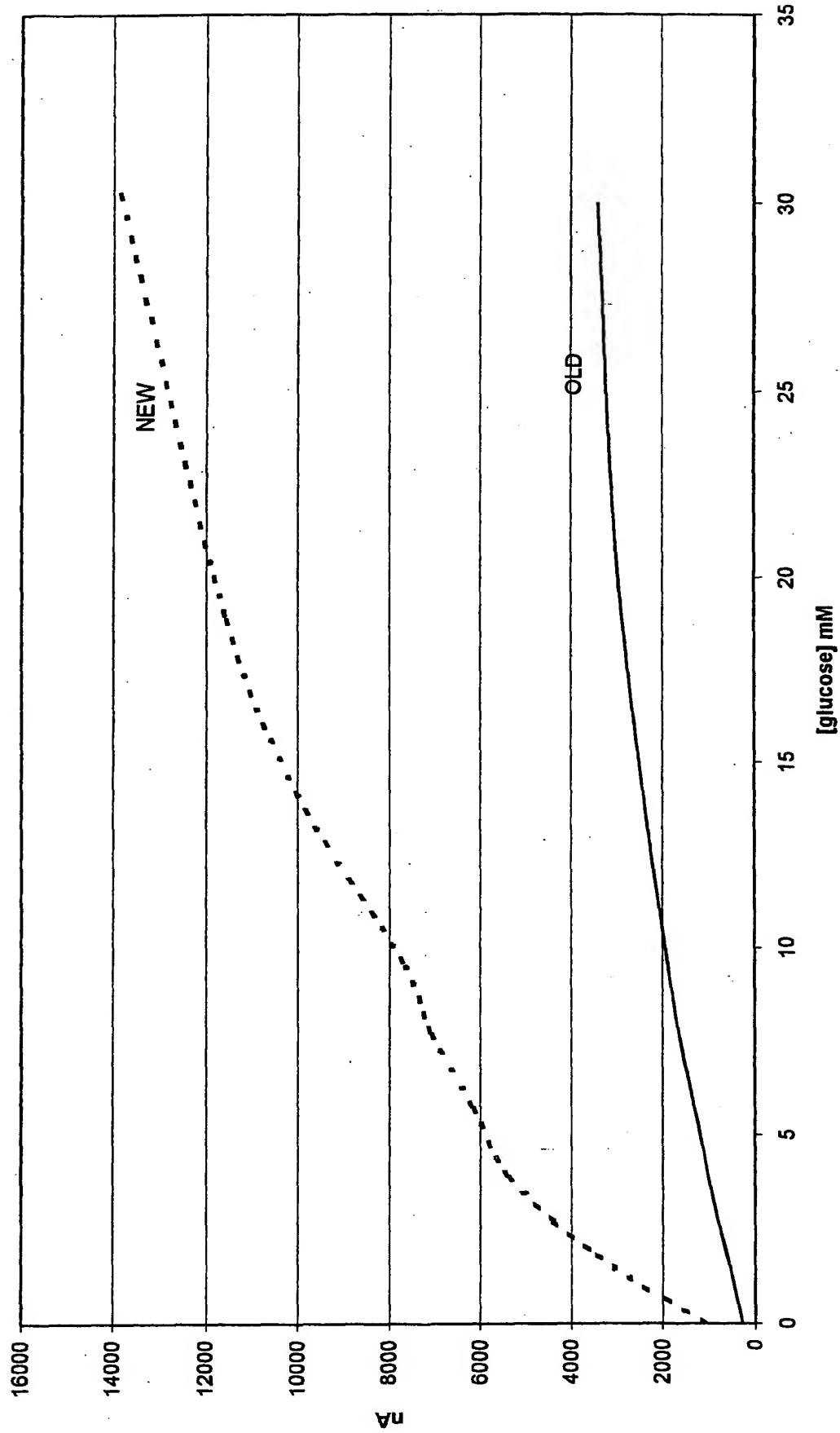


FIG. 2

Effect of Phosphate buffer concentration in top layer on response

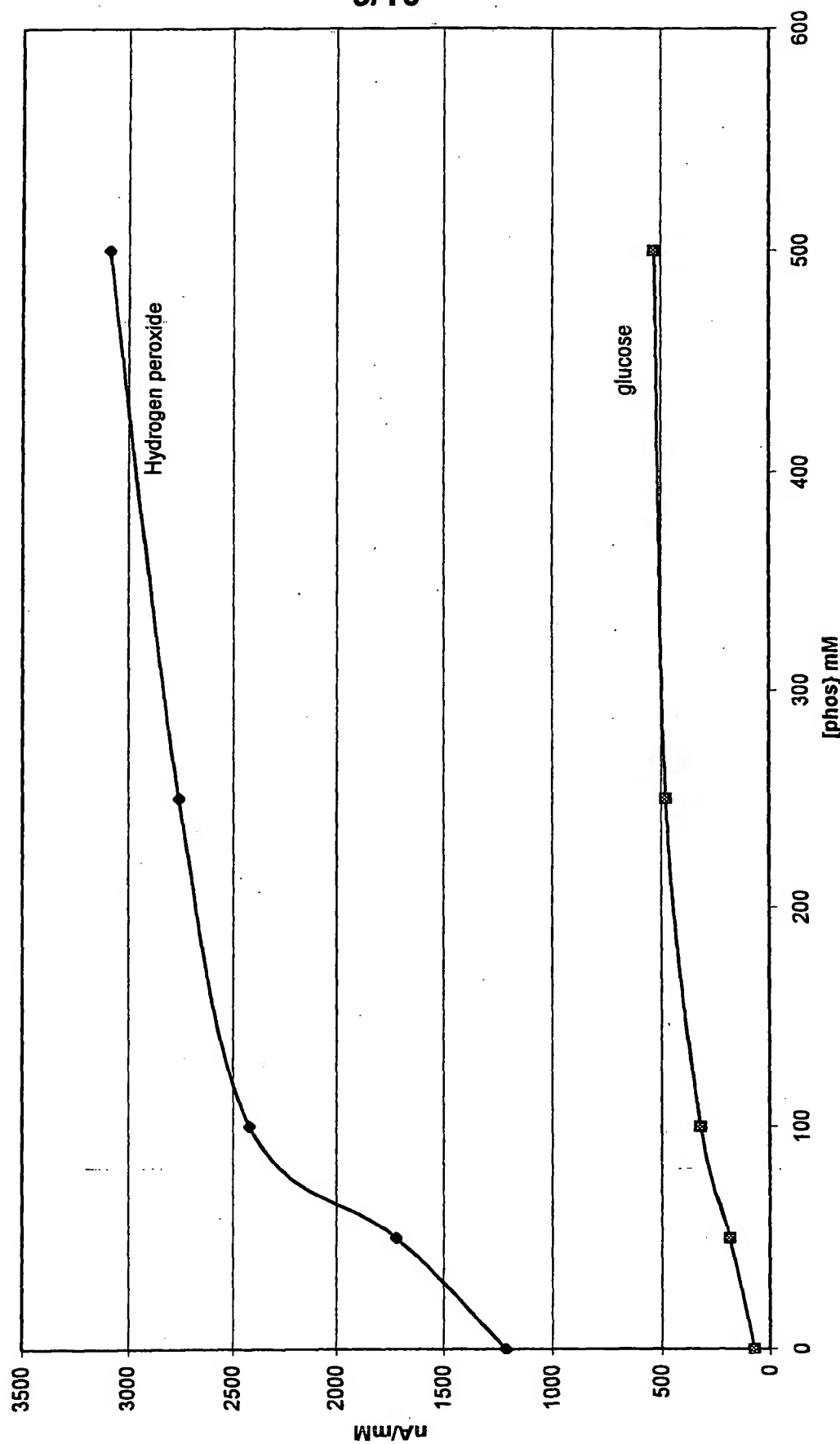


FIG. 3

Effect of pH on glucose response

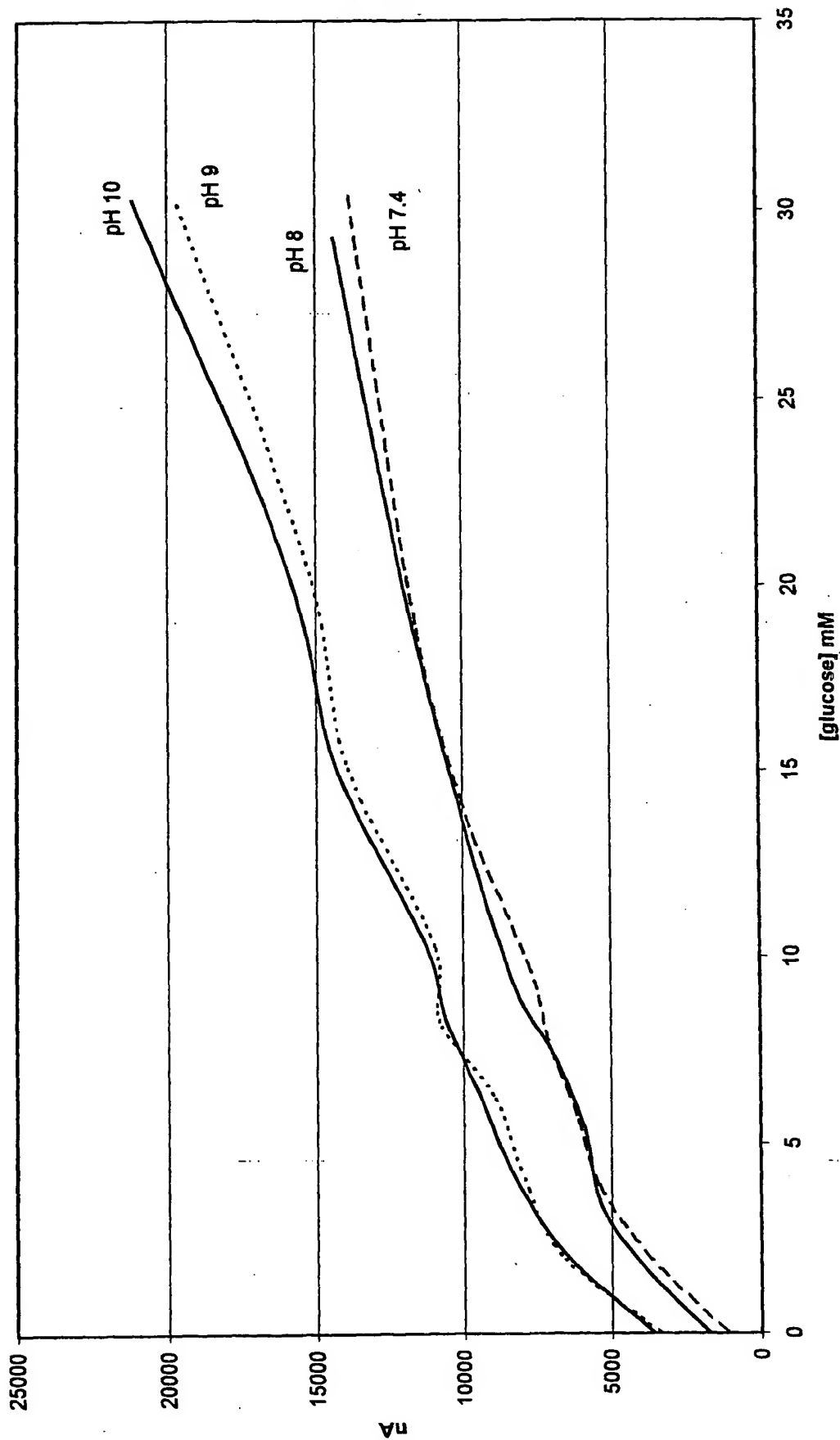


FIG. 4

Effect of pH on Hydrogen Peroxide response

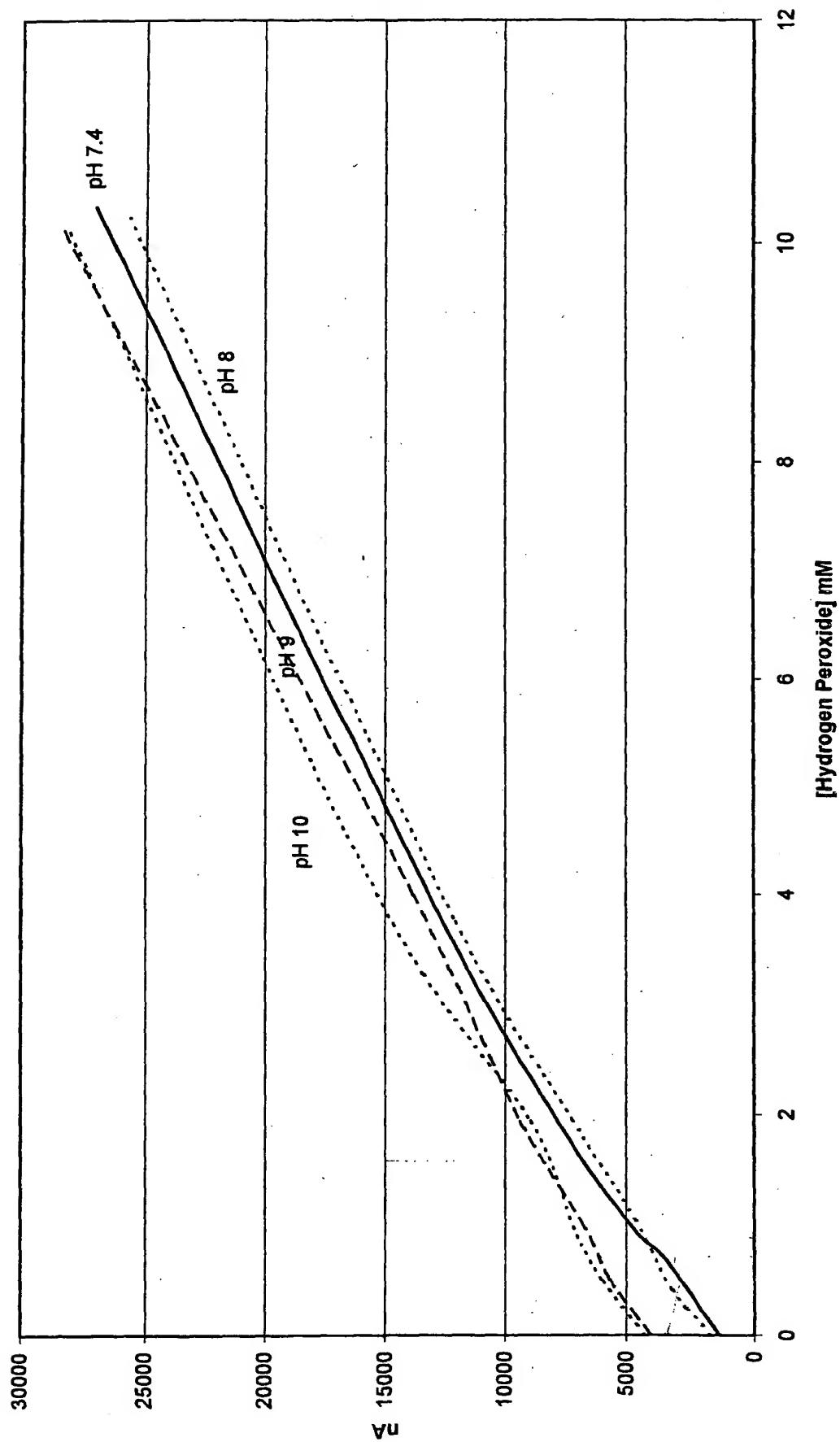


FIG. 5

pH effect on low hydrogen peroxide response

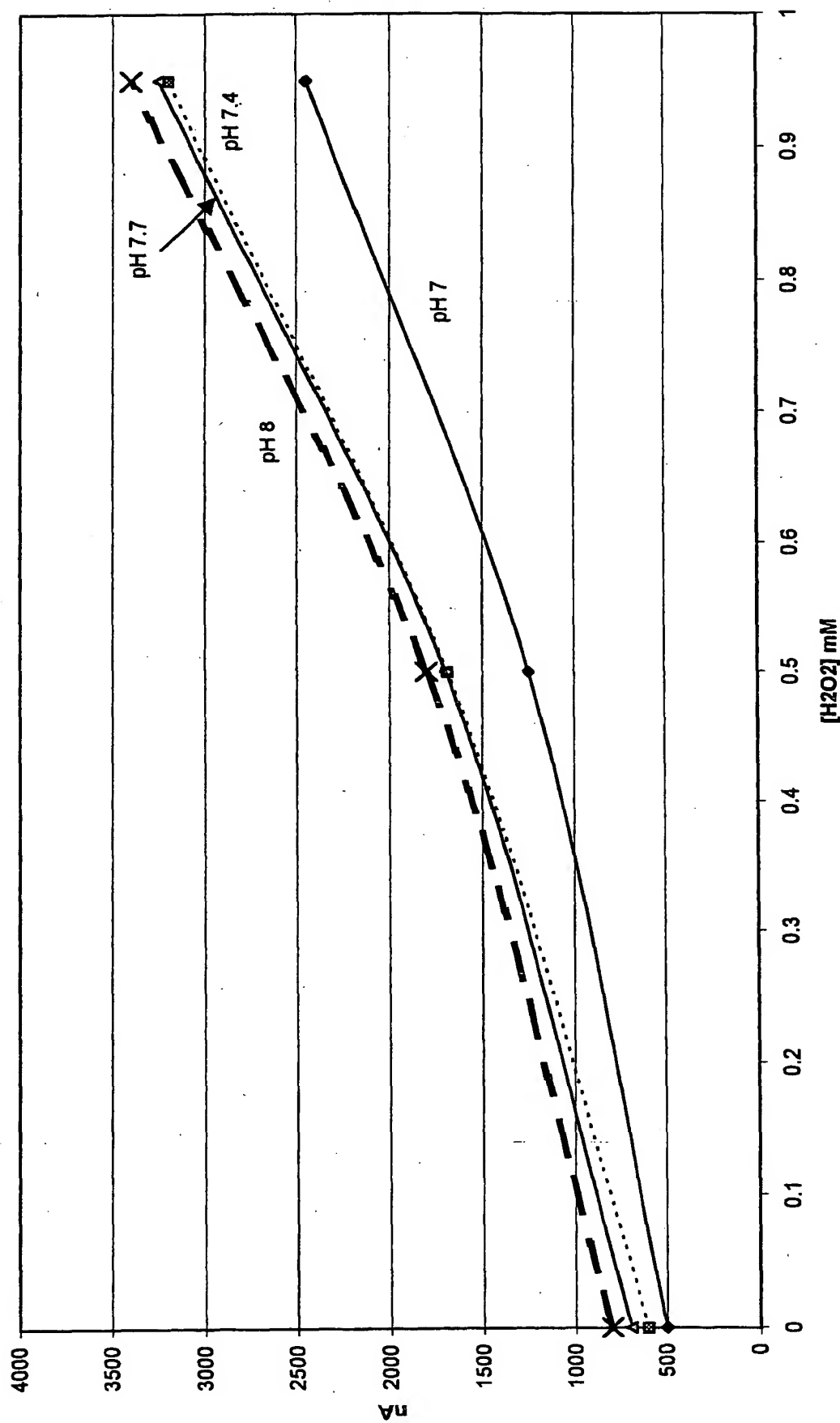


FIG. 6



Effect of buffer type on glucose response

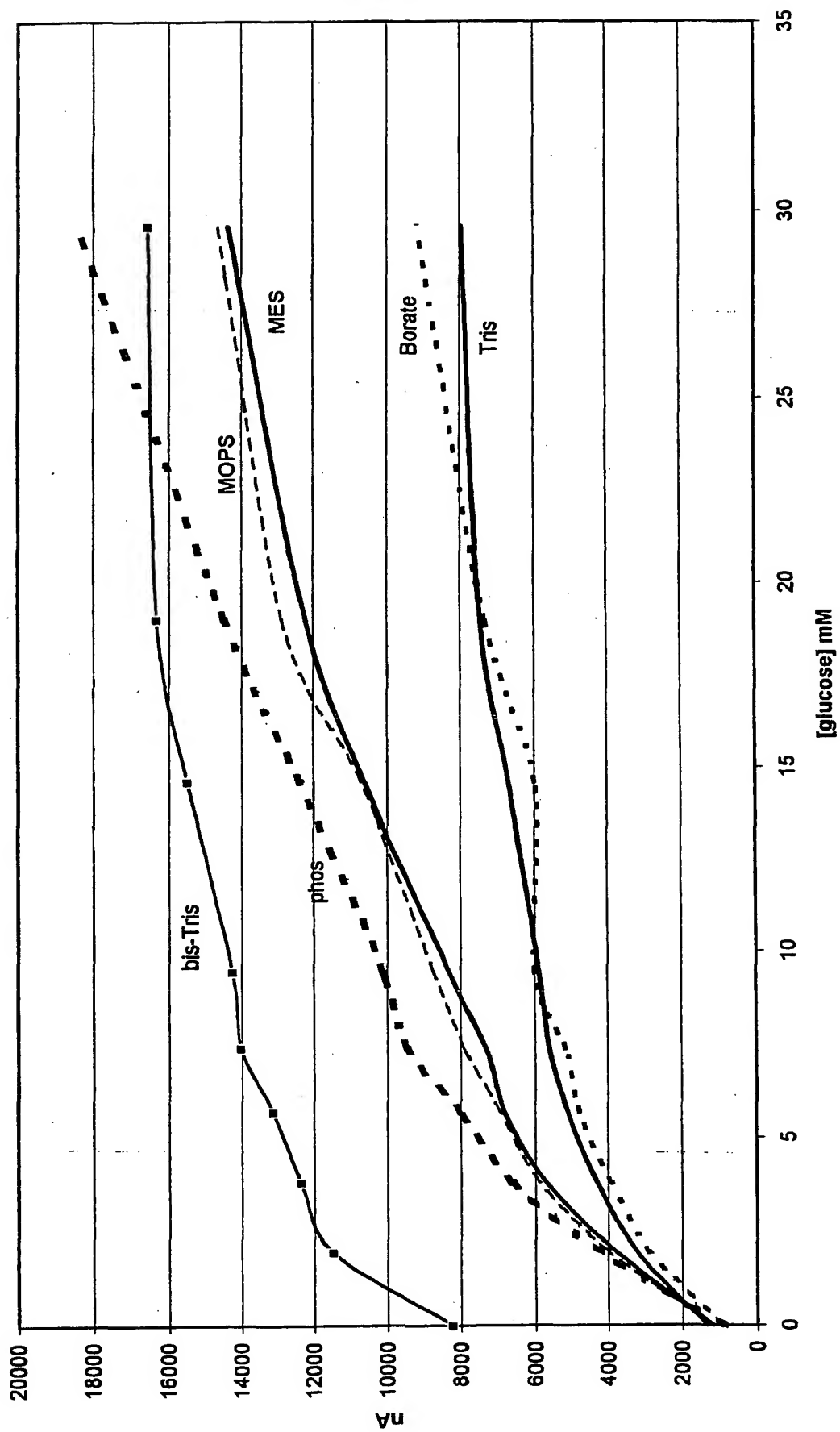


FIG. 7



Effect of buffer type on glucose response

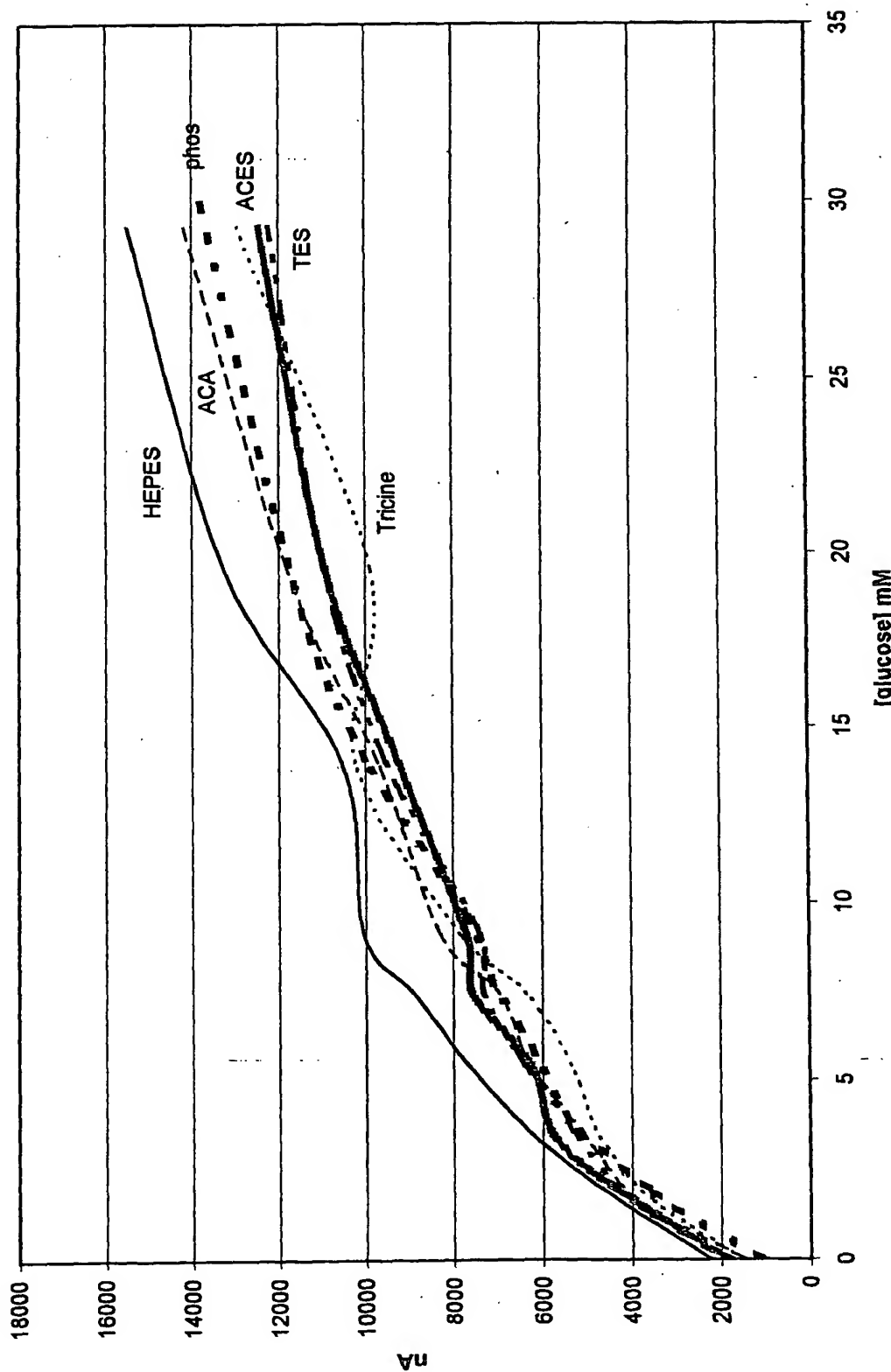


FIG. 8

Effect of buffer type on H₂O₂ response

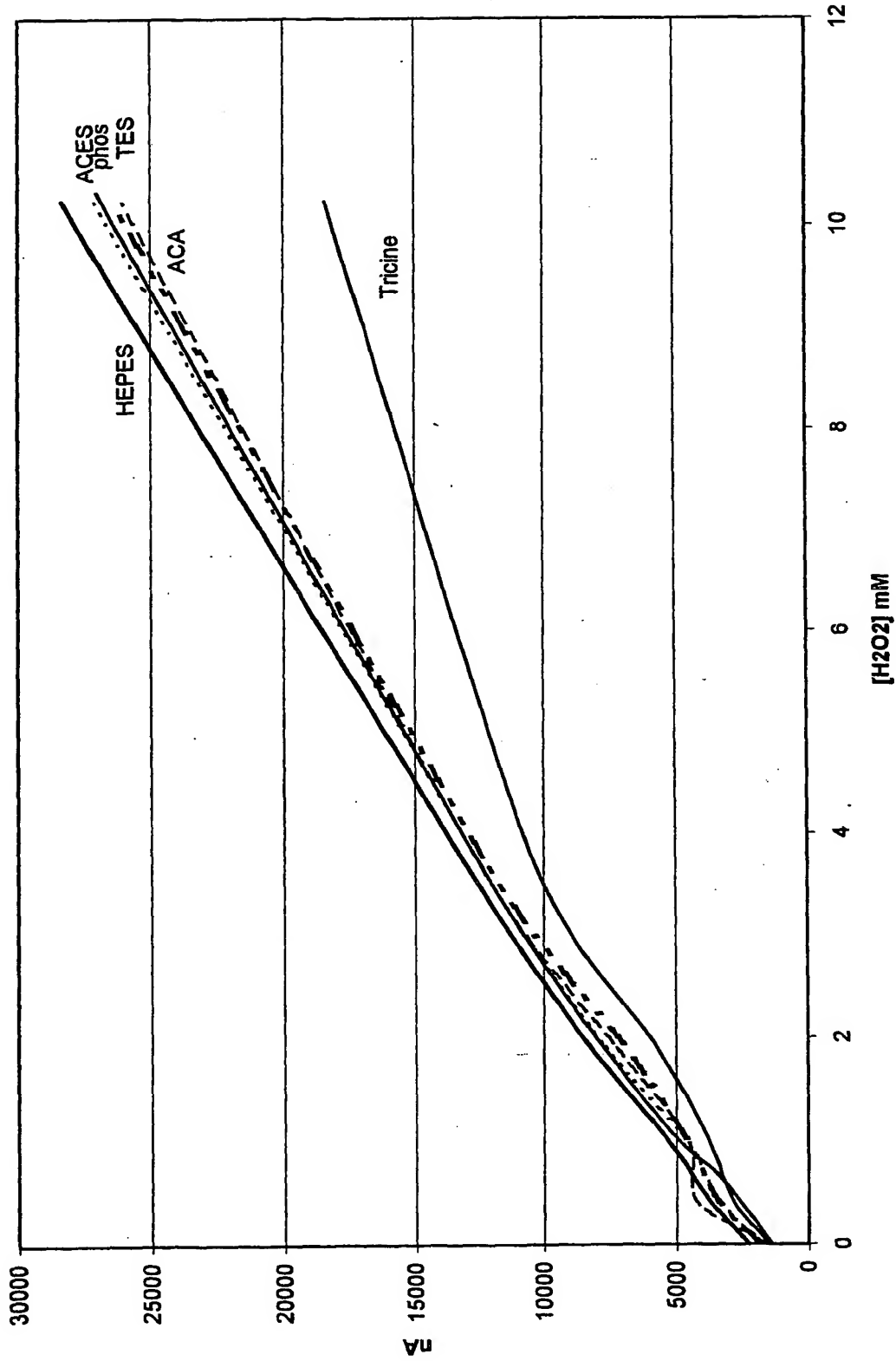


FIG. 9



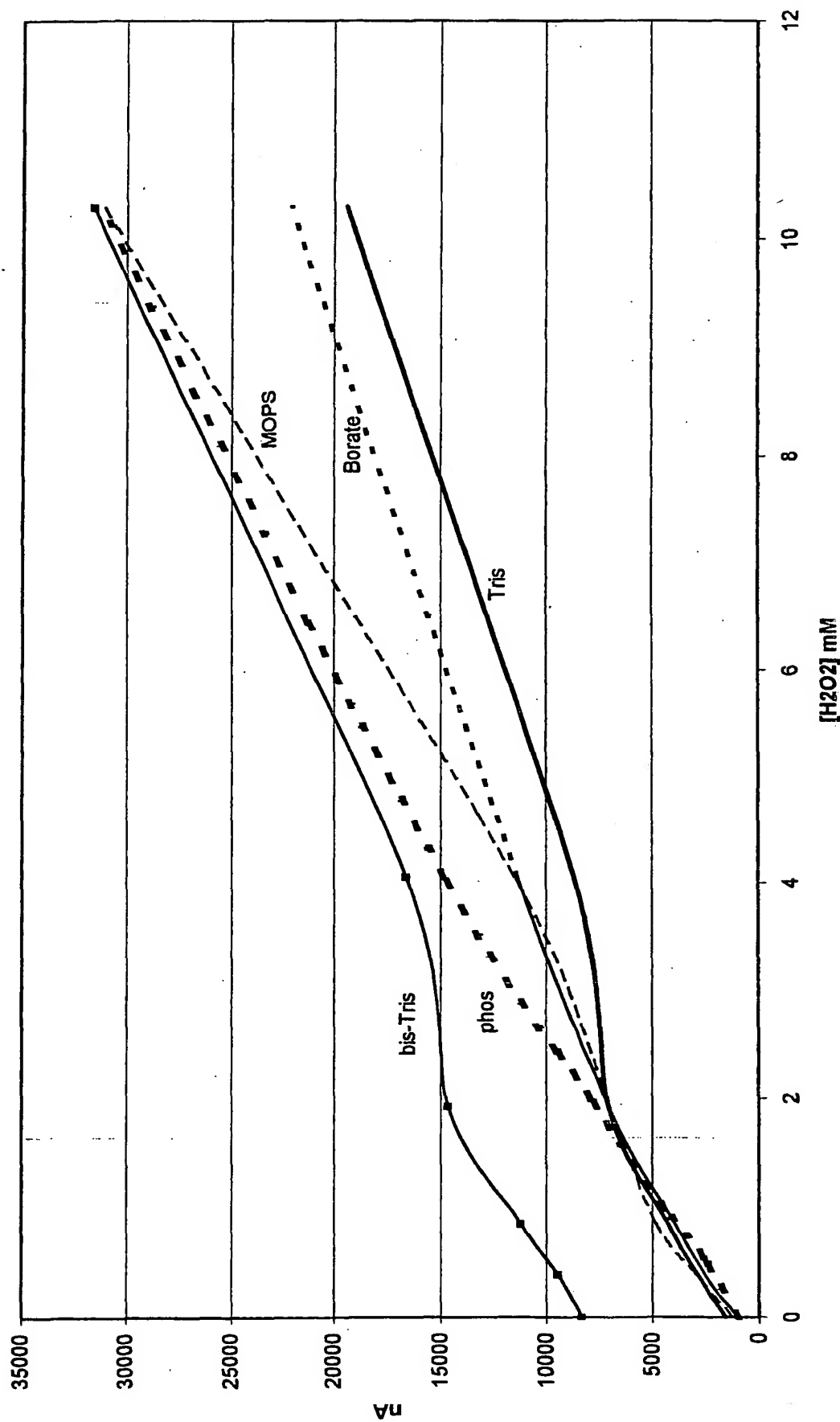
Effect of buffer type on H₂O₂ response

FIG. 10

